

Report for 2001AR3621B: Occurrence of Animal Feed Additives in Northwest Arkansas Surface Water

There are no reported publications resulting from this project.

Report Follows:

Problem and Research Objectives:

Land application of animal wastes is a common practice in Arkansas and surrounding states. Recycling of nutrients and organic matter is essential in reducing the need for fertilization and maintaining the soil quality with respect to organic matter content. Animal feeds are usually formulated with additives such as antibiotics and coccidiostats to promote growth and prevent intestinal diseases. These additives are largely excreted with the urine and feces by animals after intake (up to 90%). The levels of some antibiotics in dry poultry waste can be as high as 150 mg/kg. As a result of land application of animal wastes, additives are spread on agricultural soil, and in surface water following runoff. The consequences of the presence of additive residues in soils and water include: 1. impacting the soil fertility and agriculture productivity and therefore deteriorating the quality of the soil; 2. resulting in the emergence of antibiotic-resistant strains of bacteria and their subsequent release and spread in the environment. The danger to human and aquatic lives of transfer of the antibiotic-resistance to human pathogens may cause the serious health hazards. To date, our knowledge of occurrence and concentrations of animal feed additives in Northwest Arkansas surface water is little.

The objectives of this project are to develop an analytical protocol for selected antibiotics in water and to monitor their occurrence in surface water in Northwest Arkansas.

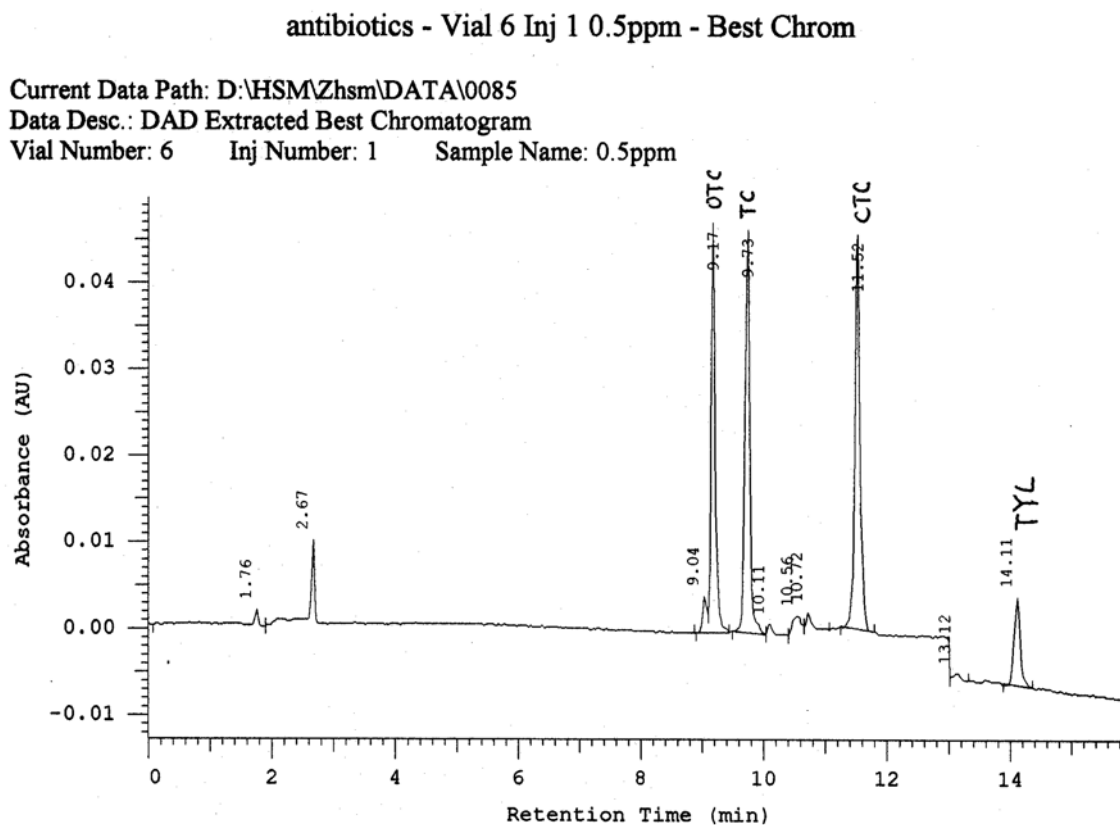
Methodology

Tetracycline (TC), oxytetracycline (OTC), chlorotetracycline (CTC), and tylosin (TYL), selected as model antibiotics, were purchased from ICN Biomedicals. A surface water was collected from a small pond in the University of Arkansas Animal Farm located in Savoy, AR. The water sample was stored in an ice-filled container, and immediately brought back to laboratory for analysis. Deionized water or surface water (100 ml each) was spiked with antibiotic standard solution (0.1 ml, ~2 mg/L each) and 0.55 g Na₂EDTA, adjusted with 0.1 mL 40% H₂SO₄ to a pH of 2.5-2.8, and rotated for 15 min to thoroughly mix samples. Spiked deionized water, surface water and spiked surface water were loaded onto and passed through HLB cartridges (Waters) that were pre-washed with 3 mL methanol, 3 mL 0.5 N HCl and 3 mL deionized water. The trapped antibiotics in the cartridge were then eluted with 10 mL methanol. Further elution did not find any antibiotics. The elutes were dried with a stream of N₂, and dissolved in 1 mL deionized water for analysis.

The concentrations of antibiotics in water were analyzed by direct injection of samples (400 µl), using a Hitachi reverse-phase HPLC fitted with UV-visible detection and a C-18 column. The mobile phase was a mixture of acetonitrile and water with a flow rate of 1.2 mL/min. The gradient system was: 0-1 min, 5 % acetonitrile; 1-15 min, 5% to 50% acetonitrile; 15-16 min, 50% acetonitrile; 16.1-20 min, 5 % acetonitrile. The wavelength was set as: 0-11.5 min, 360 nm; 11.5-13.5 min, 370 nm; 13.5-20 min, 295 nm. The concentrations of antibiotics were calculated from an external standard using peak heights.

Principal Findings and Significance

The chromatogram for the selected four antibiotics is shown below:



Clearly, with the setting we developed, these antibiotics are well separated. We calculated the recoveries tabulated in the following Tables. The recoveries are generally within the range of 50-120%. We also found that the water sample collected near the animal farm contains tetracycline and tylosin but not oxytetracycline and chlorotetracycline. These results will be highly useful in the further survey of antibiotics in Northwest Arkansas surface waters.

Table 1. Recoveries of TCs and TYL from spiked DI water.

Compound	Added/ μ g	Found/ μ g	Recovery/%	RSD/%
OTC	0.20080	0.19418	96.70	9.47
	0.02008	0.01088	54.15	9.46
TC	0.19840	0.17101	86.19	5.37
	0.01984	0.0093	46.79	0.39
CTC	0.43360	0.35961	82.70	12.27
	0.04336	0.01678	38.69	5.44
TYL	0.20600	0.17131	83.16	4.68
	0.02060	0.02019	98.04	6.35

Table 2. Recoveries of TCs and TYL from spiked fresh water and their residues in fresh water.

Compound	Added (µg)	Found (µg)	Found in blank (µg)	Residues In blank (µg/100 mL)	R (%)	Raverage (%)	RSD (%)
OTC	0.2008	0.2478	-	-	123.41	126.58	7.02
		0.2420	-		120.52		
		0.2462	-		122.61		
		0.2807	-		139.79		
TC	0.1984	0.2417	0.0474	0.05213 ± 0.0069 (13.23%)	97.93	103.25	8.58
		0.2440	0.05801		93.74		
		0.2608	0.04503		108.76		
		0.2814	0.05808		112.56		
CTC	0.4336	0.4088	-	-	94.28	104.26	9.70
		0.4222	-		97.37		
		0.4758	-		109.73		
		0.5015	-		115.66		
TYL	0.2060	0.3229	0.1931	0.2017 ± 0.0204 (10.12%)	63.01	63.39	10.59
		0.3044	0.1822		61.59		
		0.3063	0.2015		72.53		
		0.3115	0.2299		56.44		